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Thesis

Intravascular Clotting

by

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Topical Outline

Introduction

The study of intravascular clotting is filled with disagreement. Early workers looked upon the process as one of intravascular coagulation, and it is only within the last eighty years that the important role of platelets has been recognized. Today, it is known that both agglutination of the platelets and coagulation of the plasma are concerned in the formation of thrombi, but insufficient evidence of an indisputable nature, along with the inability to unify material, has saturated the entire field with confusion.

Several theories have been advanced in regard to the mechanisms of cell agglutination, but they are all more or less speculative.

The subject of plasma coagulation is afflicted with the same difficulty. Despite the establishment of a few definite and universally accepted facts, none of the numerous theories proposed to correlate these facts has met with general acceptance.

As to the ultimate cause of thrombosis, here again the search continues. Older views were concerned with mechanical causes, while more recently, biochemical and physico-chemical factors have been receiving more and more attention.

The difficulties in the way of understanding thrombosis mechanisms are due to the very complex chemical and physical phenomena involved. The only solution to the problem, authorities agree, lies in continued, accurate experimentation. The field is already flooded with theories.

I. Phylogenetic Development of Thrombosis

In the earlier days of work on thrombosis, it was thought that this and blood coagulation were one and the same. Later, however, with the discovery of blood platelets, and especially with Bizzozero's observations on their agglutination, it was suggested that perhaps the massing together of these elements was involved in the process.

With further experimentation it was soon definitely established that such was the case, and the ideas of thrombocyte agglutination and plasma coagulation became firmly associated with the study of thrombosis.

The division of these two processes into separate categories was not long in forthcoming. Several workers began investigations on certain arthropods in which fibrinogen is lacking. Thus, the agglutination process could be readily studied, without complications from the phenomena of coagulation. Tait (1920) observed that cell agglutination was the only possible process in clot formation in Gammarus Marinus. Loeb(1927), working especially with Limulus polyphemus, used an animal which contained only one type of cell, the amoebocyte, and came to the same conclusions. He found that if Limulus blood is withdrawn and allowed to flow into a dish without special precautions, the amoebocytes stick together forming a clot like material. This clot resembles grossly any normal blood clot. It consists only of the agglutinated hyalin cytoplasm of amoebocytes, and thus represents a true agglutination clot without protein deposition

on the periphery of the cells.

Another type of cell agglutination in Limulus has also been observed by Loeb(1927) which has a strong resemblance to fibrin deposition, and the resulting clot might be mistaken as one which is composed of fibrin. In this case the cells send out pseudopods, or may themselves be drawn out into long fibers. This type of agglutination was very confusing to earlier workers.

In other species of lower animals, explosive corpuscles are found, which have a coagulative function. They rupture upon exposure to the outside, and form a coagulum. This is evidenced by Hardy's studies on Gammarus and Astacus(1892).

True blood coagulation, as we know it, is first found among the arthropods in certain species of decapods. Cell agglutination is followed by true plasma coagulation in these animals, which is initiated by blood coagulins released from injured blood cells, or by tissue coagulins. These coagulins differ in their degree of resistance to injurious factors, and the tissue coagulins require more calcium for effectiveness than do those of the blood. Both show specific adaptations to the plasma, unlike vertebrate blood in which only the tissue coagulins possess this characteristic. In vertebrate and invertebrate blood, however, there is good evidence to prove that the blood and tissue coagulins are different.

Moreover, the tissue fibrinogen in vertebrates is believed by some workers to cause the transformation of fibrinogen to fibrin directly, and not indirectly by acting on a precursor

substance of thrombin, that is prothrombin. This indirect action is found in tissue coagulins in invertebrates.

The lower classes of vertebrates have nucleated spindle cells, while mammals have blood platelets to function in the clotting process.

Vertebrate blood may remain unclotted for some time outside the body, if it is protected from tissue elements. In this case, small clumps of platelets are seen by microscopic observation adhering to the surface with which the blood is in contact. This phenomenon suggests that in vertebrate blood also, agglutination of cellular elements may precede coagulation, and may occur independently of the latter. Moreover, the same factors might be responsible for inducing or accelerating the two processes.

Other experiments by Loeb(1903, 1904, 1910) in which coagulation of plasma was delayed or prevented, such as by heating to certain temperatures, also reveal that agglutination of platelets is an independent phenomenon which precedes coagulation.

Thus, it is apparent that two concurrent processes developed phylogenetically, by which organisms protect themselves against blood loss. In the first place, agglutination of cellular elements and secondly, conversion of fibrinogen to fibrin referred to as plasma coagulation. The agglutination process developed first, and is the only one present in more primitive organisms, or at least it predominates. The agglutination process was added in higher organisms, although the agglutination process is

retained, and usually initiates coagulation. Coagulation has become much more pronounced in higher forms, and usually accounts for the greater part of the clot, or thrombus.

Thus, the first stage in thrombosis, as well as in extravascular clotting, is agglutination of cellular elements, which in higher animals is followed by the increasingly important process of plasma coagulation. The following accounts of cell agglutination and plasma coagulation are therefore as important in a thesis concerned with intravascular clotting, as they would be in a thesis on extravascular coagulation.

II. CELL AGGLUTINATION

A. Theories of Cell Agglutination

That cellular elements agglutinate is a certainty. The mechanism by which they accomplish this phenomenon, however, still presents a challenge to workers in this field. Serious consideration whould be given to the possibility of the operation of more than one process.

A theory on cell agglutination set forth by Loeb(1927) seems to be closest to the truth, although it is not universally accepted. Still, it is received with more assurance than any other idea which has been proposed. According to this author, the processes leading to agglutination are like or related to those leading to amoeboid movement. In amoeboid movement there is a rhythmic softening and hardening of the ectoplasmic cell layer, probably due to the protoplasm changing its fluid content and viscosity, and it is the softening of the ectoplasm which is the essential factor in agglutination. Whatever tends to harden the ectoplasmic layers, and to remove fluid from the cells, counteracts agglutination and prevents amoeboid movement.

Although earlier ideas held that in the living normal animal the cellular elements neither stick to the walls of the vessels nor to one another, we shall see later that such is not the case. These older views stated, moreover, that physical changes, such as result from contact with rough surfaces, cause pseudopod formation and stickiness, so that the elements attach to the walls and to one another.

Silberberg(1938) has reported that, "Changes in the surrounding medium, such as variations in hydrogen and hydroxyl ion concentrations, preponderance of certain cations and anions of inorganic salts, changes in osmotic pressure of the surrounding fluid, the action of organic substances functioning as non-electrolytes all indicate that various factors affect in a similar way amoeboid movement and agglutination."

Silberberg further concludes that above a certain limit, the hydrogen ion concentration may cause hardening of the ectoplasmic layer, and separation of previously agglutinated cells, making agglutination reversible. Cations and anions may cause swelling and softening of gelatin, and thus favor agglutination and amoeboid movement. However, in too strong a concentration excess fluidity may result, and the opposite effect would be favored. Hypotonicity of the surrounding medium may act in a similar manner.

Thus, as suggested by Loeb(1927), there appears to be a definite relationship between the phenomenon of amoeboid movement and that of agglutination. Both depend upon changes in the consistency, state of contraction, and probably the fluid content of the ectoplasmic layer.

Another theory, reviewed by Silberberg(1938), bases its tenets upon studies on bacterial suspensions and agglutinating sera; it stresses surface electrical changes as the ultimate cause of agglutination. Normally, red corpuscles and thrombocytes have a negative charge which causes mutual repulsion, as

evidenced by current passage through solutions. However, it is assumed that adsorption of globulin and fibrinogen causes a loss of electric charge and consequent agglutination. The closer the isoelectric point of the particular protein approaches the neutral point, the more readily this discharge takes place, along with the resulting agglutination. The red corpuscles are less agglutinable than the thrombocytes.

This theory is not generally accepted, according to Silberberg(1938), especially since it must be supported by evidence of surface tension changes of the vascular elements. Moreover, it has definitely been established by Loeb(1927) that the surfaces of these elements are not fluid surfaces films, but are surfaces affected by gel and sol reversibility.

Two more arguments have been presented by Silberberg. In the first place, conditions are not the same in the agglutination of bacterial suspensions, and in cellular agglutination. Secondly, cellular elements have been observed to agglutinate in a different form from that in which they are present in the blood, as spindle cells or elliptic plates. They undergo a change which suggests the initial stage of amoeboid movement, and would tend to strengthen the first theory on amoeboid movement.

A third theory on agglutination reviewed by Silberberg, assumes the deposition of proteins on the surfaces of cellular elements. Serum albumins are considered especially important, along with lipoids and swollen intercellular protein substances. This theory is lacking in enough substantial evidence to warrent

its being of much importance.

Although deposition of material has been noted after plasma coagulation, it is considered to be a secondary phenomenon (Johnson, 1932). This is contrary to the accepted views on agglutination which hold that it precedes coagulation, and is an independent act in thrombosis. Another detracting feature of this theory is the accepted relationship of amoeboid movement to agglutination, and the failure to reveal any protein deposition in normal amoeboid movement.

Moriyama proposed a theory(1934) in which the colloid nature of blood corpuscle stroma was the decisive factor in agglutination. The stromatic colloid of a blood corpuscle is able to exist in an emulsoid state, only by combining with a certain amount of salt. If the salt is removed, the colloid changes to a suspension, and may be easily precipitated by cations. This change in the very nature of the corpuscles allows for agglutination.

Brief mention of serological agglutination might also be made at this time. As in ordinary cellular agglutination there are mainly theories, most of which are exceedingly speculative, due to the invisibility of the antibody molecules. For example, the "Lattice" theory (Boyd and Hooker, 1938) assumes that antigen molecules, or elements alternate with antibody molecules forming long chains or links. Many chains supposedly interweave, and the formation continues as long as there is a supply of antigens. An older view holds that the antigen becomes coated with antibody molecules. Then, there may be either a simple lack of

repulsion between the coated particles, leading to agglutination, or there may be some sort of an attraction of the coated particles.

Recent work on sludged blood deals with the presence of agglutinated cells in plasma, and will be discussed in the following section.

B. Sludged Blood

The discovery of this material is relatively new, and workers believe that it will aid in the solution of many problems regarding blood coagulation. It was actually observed earlier, but its importance was never realized.

The term was employed by Knisely and workers(1942, 1945, 1947) to describe circulating blood in which cells are agglutinated into masses, thus changing the relative fluid state of blood to a more "slushy" condition. The cells in this case are not in the form of a rouleaux, and are coated with a fibrin-like precipitate.

Fahraeus(1929) had observed an intravascular aggregation of red corpuscles in diseased persons and pregnant women, differing from rouleaux formation. In rouleaux formation there are usually many more corpuscles, and they are more clearly and regularly united with each other, while the aggregations described by Fahraeus are much more clustered, with large lakes of free plasma separating the clusters. Also, rouleaux fall apart very easily when fluid currents are set up in microscope preparations by exerting slight pressure on the cover glass. This situation is not found in the pathological aggregates.

Knisely and workers(1947) found sludged blood only in unhealthy animals and men. Mild cases of intravascular coagulation have been found where sinusitis of various degrees of severity, and other upper respiratory system malfunctions are endemic. More severe degrees of sludged blood occur only where

persons are ill enough to need the assistance of a physician. No severely ill person has yet been observed by Knisely, who was not afflicted with this condition, along with visibly pathologic vessel walls.

Earlier work had been done by Hayem as far back as 1889, he observed "--that the blood in inflammatory diseases, besides being rich in fibrinogen, is also characterized by an increased tendency of the thrombocytes to agglutinate."

At the present, a careful investigation of sludged blood is being made by Knisely and workers. They believe that the agglutinated red cell masses may be cemented together upon settling out of the plasma, and may actually be a major factor in thrombus formation.

In 1947 they observed that when first formed, the outer surfaces of the precipitated material is sticky, and contact of masses results in larger masses. The agglutinated red cell masses tend to settle out of the plasma, mostly in vessels which are horizontal or nearly so, and especially in those wherein the blood must flow slightly uphill.

As the sludged blood flows along there is a decreased rate of speed from the center of the vessel to the periphery. When the masses become heavier than the fluid, and the flow is slow enough, sedimentation will occur. Above this critical speed, however, the masses are rolled up into the axial stream and constantly swept along.

The sedimented masses in some cases may remain free for

an hour or two, regardless of how packed they may be. Then they may be either knocked loose by another mass, or blood current to join the regular circulatory flow, or the packed masses may slowly become cemented together, forming long gelatinous masses, which narrow the lumen of the vessel.

Knisely, Eliot and Bloch(1945) believe that the sludge initiator substances might be related to the substances capable of initiating blood clotting. They observed that injury to a monkey omentum caused sludge formation in local vessels, and from here the sludge was carried into the general circulation.

They also observed that white cells, which are known to travel close to the endothelium, attach at the location of the injury, often several layers thick, and pieces are torn off by red cell masses as they float by. This restores many of the white cells, which had gathered at the injury, to the general circulation.

Swindle(1937) stated that a slight temporary agglutination of erythrocytes in various systemic capillaries and veins is a normal physiological phenomenon. This agglutination, however, becomes exaggerated and dangerous under certain conditions in normal animals, and especially in animals which are not normal. De-agglutination may fail, resulting in the incipient stage of thrombosis.

He further reports that carbonic acid is the principle agglutination factor normally present in blood, being forced into capillaries by skeletal muscle activity, along with other

catabolites. Some unknown agglutination factor, which is present in pressor-depressor animals, and absent in depressor animals, then causes erythrocyte agglutination with the assistance of the carbonic acid.

Plasma will finally wash these cells free of the agglutination factors, and either complete or incomplete de-agglutination will take place.

C. Classification and Description of Thrombi

There are three general types of thrombi: agglutination thrombi, composed of cellular elements; combination thrombi, composed of both elements and also fibrin from coagulated plasma; and fibrin thrombi, entirely composed of fibrin. The second type is by far most common. The distinction between agglutination and fibrin thrombi was clearly shown by Rabinovitch (1929), who found that ox serum injections in rabbits produced agglutination thrombi, while dog serum injections produced fibrin thrombi; also, Shionoya, Johnson and Rountree(1927) experimentally produced white thrombi composed of platelets and leucocytes. They used hirudin and heparin, and obtained thrombi completely free of fibrin.

1. Agglutination Thrombi

Agglutination thrombi are composed of thrombocytes, erythrocytes, platelets and combinations of these elements. They are further subdivided according to the exclusiveness or dominance of one of the cell types entering into the clot. There are platelet thrombi, white corpuscle-thrombi, red corpuscle-thrombi, and mixed thrombi. Actually, a pure thrombus of any of these types is a rarity in more highly developed species, although one element usually dominates in the clot. Platelet thrombi consist essentially, if not completely, of agglutinated thrombocytes, or spindle cells in lower vertebrates.

Heat has been shown to agglutinate spindle cells in the vessels of the tongue and mesentary in amphibia, according to Zahn(1875), while thrombocytes have been agglutinated by mechanical irritation, or by introduction into vessels of "wettable" foreign bodies in mammals. The latter occurs much more efficiently if the blood flow is slightly retarded, with the foreign objects serving as bases for agglutination.

Mechanical irritation of vessels is accomplished by applying irritating chemical compounds to the outside wall, thus injuring the endothelium, and creating favorable conditions for thrombocyte agglutination. The same result will be produced if these substances are applied intravascularly, without injury to the vessel walls upon administration.

White cell-thrombi should not actually be classified as a special type of thrombus. White cells are very often found in the other thrombi groups, but no pure or essentially pure thrombus of these cells has ever been found. Combinations of white cells and thrombocytes are sometimes considered as white cell thrombi. Aside from a passive inclusion, white cells may enter thrombi via chemotactic influences. Mononuclear white cells participate in blood clot organization, while the polymorphonuclear cells probably aid in clot liquefaction.

Red corpuscle thrombi are chiefly composed of agglutinated erythrocytes, and their formation may take place without the accompaniment of platelet or white cell thrombi, more easily

than the vice versa condition.

Platelet or white cell thrombi depend upon amoeboid movement for formation, while red cell thrombi are the result of certain chemical substances acting on erythrocytic surfaces. Thus, there are two different processes involved in the production of these two types of thrombi.

Various experimenters have induced red cell-thrombi. Loeb, Strickler and Tuttle(1910) obtained such thrombi by injecting ox-serum intravenously into rabbits with and without hirudin, as Rabinovitch(1929) had done. Rabinovitch's agglutination thrombi were also of this type. The chemical effect of the agglutinins in the rabbit blood causes the red corpuscles of the ox-serum to agglutinate.

Mixed agglutination thrombi are the most common, containing some of all the element types, if not all. White thrombi are the determining and most dependable factor in thrombosis, with the red thrombi usually of secondary importance. Most thrombi have a white core, then a mixed layer, and finally a red corpuscle surface.

2. Combination Thrombi

Combination thrombi consist of agglutinated elements and fibrin, and are more commonly found than either of the other pure types. There is almost always a base of agglutinated cells upon which the fibrin deposits, and it is usually this fibrin covering that makes up the greatest part of the thrombus.

3. Fibrin Thrombi

Fibrin thrombi are composed solely of fibrin, as the result of plasma coagulation. As in the case of the pure agglutination thrombi, this group is not usually found in the circulation, but such thrombi have been produced experimentally. A different mechanism is involved from that which causes agglutination thrombi.

Hemolytic agents can produce thrombi, as reported by Loeb, Strickler and Tuttle(1910), and further experimentation has shown that if hirudin is introduced at the same time, no thrombi form for at least some time. The thrombi which finally do form are made up of pure fibrin with some entrapped cellular elements.

Grossly, fresh thrombi appear round in shape, and of varying lengths, when free or only slightly adherent to vessel walls. The shape of attached or old thrombi vary.

Thrombi can be attached by the grip of their white cells on the endothelium, or by the action of cells which penetrate into the thrombus from surrounding connective tissue(Silberberg, 1938). Growth processes from these cells, and from the lining vascular endothelium, then form a closer connection between the thrombus and vessel wall.

A thrombus may show either a laminated or a uniform structure. Different colored layers alternate in the case of the former, and this arrangement supposedly results from a slow clotting process, during which there is only partial bloodflow.

The term pulsation thrombus is used to describe this type. A uniform clot results from acute stoppage of the blood-current, or stagnation in the bloodflow. A stagnation thrombus results.

White thrombi have Zahn's markings on them. These are small, ripple-like, net-like and linear markings, which are only seen in the white part or core of a completed thrombus. They fade and disappear as the red portion is reached. The explanation given for them has been that they are the effect of to-and-fro movements of waves of blood on the platelets.

Another view differs only slightly by suggesting that the blood does not move the platelets from their original position, but actually arranges them in their final form.

III. PLASMA COAGULATION

Two established facts stand out above the conglomeration of disputed material in the field of plasma coagulation. These facts are:

1. The formation of thrombin from its precursors: prothrombin, calcium, and the tissue factor (thromboplastin).
2. The conversion of fibrinogen to fibrin under the influence of thrombin.

These facts, which constitute the thrombin theory of coagulation, form the cornerstone upon which most modern theories of coagulation have been raised.

The more important agents in blood coagulation will now be described, after which some of the non-thrombin and thrombin theories will be reviewed, at the risk of repetition.

A. Thrombin, Fibrinogen, Fibrin

Thrombin is simple protein in nature, and is made up of more than a single component, as determined by solubility tests and electrophoresis apparatus. Thermal denaturation can begin as low as 40 degrees C., with complete destruction at 50 degrees C. in five to ten minutes. The significance of sulfur, which is probably present in SH groups, and a carbohydrate fraction, is unknown. Pure thrombin contains only minute traces of radioactive phosphorus, and there is much disagreement as to the presence or absence of calcium. It is very readily soluble in pure water, and is completely precipitated unchanged by half saturation with ammonium sulphate.

Thrombin gradually loses its ability to coagulate fibrinogen, if allowed to stand in solution for long periods, even if protected from putrefaction by chloroform. This degeneration of function is increased at higher temperatures. It may be kept indefinitely if dried at low temperatures and protected from moisture in a desiccator.

The manufacture of thrombin yields a relatively pure product. Modern preparation is via prothrombin, for the most part. Also, two other methods are sometimes used, which formerly were the standard means of obtaining this substance. It might be extracted from a fibrin clot upon which it has been absorbed, by washing with sodium chloride. This thrombin is free from admixed protein. Also, serum is precipitated by alcohol, and the precipitate, after standing under alcohol for some time, is dried and

the thrombin extracted with water.

Thromboplastic material forms some thrombin when present in the circulating blood. The thrombin forms quickly, and is combined rapidly with an antithrombin, equilibrium soon returning to the system. Such rapid inactivation aids greatly in maintaining normal blood fluidity. This same procedure also takes place in the normal process of coagulation. Thus, according to Gasser(1916), thrombin has two normal fates in the natural process of coagulation. It may either be combined with antithrombin or fibrinogen. The former would be detrimental to coagulation, while the latter would assist it.

Thrombin in serum may either be formed from prothrombin by the action of thromboplastic substance and calcium, or may be liberated from the thrombin-antithrombin complex by alkali activation. The thromboplastic substance will not liberate thrombin from the complex.

Fibrinogen is a protein belonging to the globulin group. If blood plasma is heated to 56 degrees C., the fibrinogen is removed as a heat coagulum, and the power of clotting or fibrin formation, is lost. It exists in the plasma as a separate entity as shown by electrophoresis apparatus, and makes up 3 to 7 per cent of the plasma proteins. It is the least soluble of all blood proteins.

Mills and Guest(1921) have shown that there is also a tissue fibrinogen, which can clot blood directly, unlike the ordinary tissue factor. It is considered to be a thromboplastin,

nevertheless, and will be described more fully under a discussion of the tissue factor.

Jones and Smith(1930) have presented good evidence to indicate that the liver is essential to fibrinogen formation. They found that its concentration dropped about 20 to 50 per cent in 12 to 20 hours in hepatectomized dogs. Also, they have indicated that the use or destruction of fibrinogen in the body is variable, and might increase under certain abnormal conditions. Its average normal level in human plasma is 250 mg. per 100 ml.

Other workers have been unable to ascertain its functional activity in the body, or to discover what organ or organs utilize it, although a few observers point to the lungs as being important in its metabolism. Fairly conclusive material has been presented, which argues against fibrinogen being formed in the bone marrow, as previously thought(Drinker and Drinker, 1916).

Edsall and workers(1944) determined the concentration and solubility of fibrinogen by standard techniques in plasma fractionation. The protein is precipitated by various neutral salts such as sodium chloride or ammonium sulphate.

Fibrinogen can be manufactured in a relatively pure condition, but its preparation is especially difficult. It denatures very easily, is hydrolized by proteolytic enzymes such as tryptase, or is clotted to fibrin by small amounts of contaminating thrombin.

Molecules of fibrinogen appear to be miniature fibrils that are much longer than molecules of serum proteins, but are about

the same thickness. Although relatively long, the fibrinogen molecule is much smaller than the microscopically visible fibrin threads, and both are alike in their molecular characteristics. From studies with the dark field electron microscope it is further believed that individual fibrin molecules are fine fibrils which aggregate into larger units or micelles within the fibrin needles(Edsall & workers, 1944). Very recently(1947), Hawn and Porter have observed cross striations on individual needles. These fibrin needles are delicate elongate structures, which fuse to form a meshwork. Many formed elements become entangled, and the whole mass has the appearance of a crystalline gel. Thus, fibrin is the immediate cause of clotting.

This gel is probably not, as presumed by some men, an inclusion of a liquid phase within a solid confine. The gel character supposedly is due to the surface action of the fibrin needles upon the water, not to the surface tension in the liquid between fibrin needles(Howell, 1916).

Eagle(1934) removed all the calcium from a thrombin solution, and its coagulating activity was not affected. This oxalated thrombin then clotted a good sized fibrinogen solution, resulting in the formation of fibrin of very noticeable weight. It was then calculated that if there was any calcium in fibrin it would of necessity have an impossible molecular weight, or what is equally impossible, one molecule of calcium would have to combine with 50 to 100 molecules of fibrinogen. Thus, it is

quite improbable that fibrin contains any calcium in its molecules.

There is a high degree of specificity in the relationship of thrombin to fibrinogen. No other protein is known which may be affected in a similar way. The actual nature of the reaction between thrombin and fibrinogen is still unsolved, although several theories have been proposed. The idea that thrombin acts as an enzyme has been favored on the basis of available evidence, but no conclusive facts have been set forth in this regard.

Some few workers, disregarding the thrombin theories of coagulation, assume that thrombin is merely a by-product in this process. More convincing work, however, has disproved such an idea, and as already stated, the importance of thrombin is very generally recognized.

That fibrinogen is then transformed or converted to fibrin, is also taken as fact. Here too, however, workers have been unable to agree upon the exact nature of the action. Hammarsten(1880) believed that thrombin might act as a hydrolytic or proteoclastic enzyme, which causes cleavage of the fibrinogen molecule, and thereby produces insoluble fibrin and a soluble serum globulin. Although most workers agree with this idea of fibrinogen conversion to fibrin being the result of hydrolysis, experimental evidence of any sufficient calibre is lacking.

Hammarsten(1883), himself, later turned from this theory when he discovered that variable amounts of fibrin are formed from a given weight of fibrinogen. He later took up the idea

that there is a molecular change from fibrinogen to fibrin.

A speculative theory set forth by Hekma(1914) considers fibrinogen to be an alkalihydrosal of fibrin dispersed as amicrons, and further implies that coagulation is the result of an unexplainable, increased acidity in shed blood. Thrombin then takes part in agglutinating these amicrons in crystal or needle form, after being liberated from platelets and leucocytes, probably by the increase in hydrogen ion concentration. This agglutination is preceded or accompanied by an independent act of dehydration. The transformation to fibrin was further considered by Hekma to be a reversible sol-gel transformation, with the fibrin capable of being changed back to fibrinogen, by dissolving it in alkali.

This whole idea is quite obsolete now, especially since Barkan and Gasper(1923) showed that an alkaline solution of fibrin is not a solution of fibrinogen. The former cannot be coagulated a second time by the addition of thrombin.

Furthermore, this theory, and several other theories on fibrinogen coagulation, have been challenged by the work of Eagle and Baumberger(1934). These men reported no significent hydrogen ion shift during the fibrinogen conversion, whereas such a change was essential to many theories. This problem, along with many others, still remains undecided.

A later view by Hekma(1931) suggests that some, if not all of the fibrinogen, exists in the circulating blood in a

complex also containing globulin, albumin, and thrombin or prothrombin. Kinase from tissue cells or platelets then dissociates this complex, and the free fibrinogen deposits as fibers or needles which form a gel. He conforms to the essential nature of irreversibility in the normal clot, by assuming the presence of a cement from the erythrocytes, which binds the fibrin fibers and needles.

Del Baere(1932) contributed the idea that an electrical effect might be the basis of the reaction. By cataphoresis experiments he showed that thrombin carries a positive charge. If fibrinogen is then assumed to possess a negative charge, fibrin deposition may be explained by a flocking out of fibrinogen after its electrical adsorption of thrombin.

Of course, a similar reaction should occur between thrombin and negatively charged albumin or globulin, but Del Baere states that only a change in osmotic pressure will result from such a combination.

Stuber and Lang(1930) believe that fibrinogen is flocked out at its iso-electric point in clotting, by increased lactic acid and resulting hydrogen ion concentration, from the glycolysis of blood sugar. The blood clot is believed to adsorb the hydrogen ions, and the free fluid is thus left alkaline. Verification of this latter fact has given some support to this theory.

The idea of a chemical union was set forth by Fischer (1933). He considers thrombin to be a calcium lipoid protein,

which causes the formation of fibrin when chemically united with fibrinogen. The calcium supposedly adds on to the fibrinogen and forms insoluble fibrin. Loucks and Scott(1930) considered thrombin to be a calcium-"Tissue Juice" compound which alters the sol fibrinogen to the gel fibrin.

The preceding ideas have been strongly refuted, since active thrombin has been prepared from decalcified serum. Also, prothrombin has been activated to thrombin in the absence of calcium, by other reagents such as chloroform or alcohol. It must be remembered, however, that the issue concerning the presence of calcium in thrombin has not been definitely decided.

Mellanby(1908) believes that fibrin ferment is responsible for the coagulation of fibrinogen solutions. He states that fibrinogen is always associated with prothrombin. The tissue factor and calcium then act upon the prothrombin to generate fibrin ferment, which is the actual coagulant. It may be obtained experimentally upon coagulation of fibrinogen solutions by the tissue factor and calcium. The fluid expressed after complete coagulation has coagulative properties and is called fibrin ferment solution. This fibrin ferment is actually thrombin, and the antifibrin-ferment might be anti-thrombin.

Lozner, Kark and Taylor(1939) prepared plasma euglobulin from plasma which had been deprived of fibrinogen and prothrombin. Previously, Lozner and Taylor(1939) had shown that the euglobulin portion of active globulin was associated with the coagulative ability. Thus, it appeared quite evident to them that there was a constituent of normal cell-free blood plasma, which could clot blood independent of platelets, prothrombin or fibrinogen, in the presence of calcium.

Attempts to reconcile this with other known facts on coagulation have failed, although several other workers have presented similar evidence, including Howell, who has called the substance "Plasma thromboplastin."

Another protein fraction, a pseudoglobulin fraction with coagulative properties, has been found in rabbit's blood by Parfentjev(1941). Actually, this pseudoglobulin fraction is thrombin. It was found to act directly on fibrinogen.

Baumberger(1941) believes that the long crystals of fibrin that compose a clot are the result of molecules lining up, because of the formation of bridging S-S groups produced by oxidation of the SH groups of the fibrinogen by thrombin.

Robbins(1944) has demonstrated what he believes to be two different fibrins, based on their solubilities in dilute acids and alkalis. He believes that the first fibrin, T-fibrin, forms from fibrinogen plus thrombin, and then ionic calcium plus a serum factor are necessary for its conversion to Ca-fibrin.

Mommaerts(1945) believes that the clotting of fibrinogen

by thrombin is caused, partly at least, by a coacervation process which is due to electrostatic attraction between both positive and negative groups. He found that on the acid side of the isoelectric point of fibrinogen, a substance called profibrin is formed, which is a compound of thrombin and fibrinogen, resulting from a primary reaction between the two. This is partly confirmed by the fact that at pHs favorable to clotting, fibrin is negatively, thrombin positively charged, whereas profibrin has both negative and positive charges. Electrostatic attraction between the positive and negative charges takes place in the second step, which is the polymerization of profibrin to fibrin.

B. Prothrombin

Prothrombin is considered to be a protein, but there is no really decisive proof of its chemical nature, since there is no evidence that it has ever been obtained in very pure form. Various reactions which it has been shown to undergo might be the result of accompanying impurities.

It is not present in great concentrations in plasma, its yield never exceeding 0.6 per cent of the total plasma proteins. Most observers believe that its thermal denaturation takes place at about 62 degrees C. However, enzyme-free preparations are more stable, and retain their ability to produce thrombin when activated, even after being boiled for several minutes.

Quick(1943) has indicated experimentally that prothrombin might be composed of calcium and two separable components, A and B. The A component is heat labile, specific to some degree, and is destroyed by oxidation in oxalated plasma. It is stable in the intact prothrombin complex. The B component is also heat labile, and stable in the complex. It is adsorbed from oxalated plasma by aluminum hydroxide, but not from unchanged plasma.

Great difficulty isencountered in attempting to obtain pure prothrombin. One reason for this is the easy conversion to thrombin during purification. Also, prothrombin is made unstable, possibly due to the digestive action of serum tryptase (an accompanying enzyme), calcium salts, cephalin (phospholipid), and thrombokinase (tissue factor).

There are two general methods for preparing prothrombin.

Either prothrombin is precipitated out by some reagent, or the fibrinogen is precipitated out, leaving prothrombin in solution.

Specifically, prothrombin may be prepared from blood plasma by the following means:

1. Mellanby(1908) used iso-electric precipitation. After diluting oxalated plasma with twenty volumes of water he precipitated it by adding dilute acetic acid. Prothrombin is then dissolved out of the precipitate, which also contains fibrinogen and serum globulin, by dilute calcium bicarbonate. Dilute acetic acid is then used a second time to precipitate out the prothrombin from the calcium bicarbonate solution.

2. Bordet and Delange(1913) treated oxalated plasma with a suspension of tricalcium phosphate, and suspended the washed precipitate in a salt solution where it was to be submitted to the action of a stream of CO_2. The CO_2 dissolved the phosphate, and left the prothrombin in solution.

3. Howell(1914) precipitated small 5 c. c. lots of oxalated plasma by an equal volume of acetone. After rapidly filtering off the precipitate and washing it with ether, it was spread in a thin layer on the filter paper and dried in the air current from a fan. Cekada(1926) obtained a prothrombin solution by placing strips of the dried filter paper in 10 c. c. of distilled water which contains two drops of .5 per cent solution of sodium bicarbonate. The prothrombin solutions are prepared fresh when needed. This method is one of the most efficient and reli-

able known. The solution is free from other fibrin factors with the possible exception of traces of anti-thrombin.

4. Cekada also tried fibrinogen removal. In this method, oxalated plasma is heated in a water bath from 55 to 56 degrees C. The precipitated fibrinogen can be centrifuged off. The only trouble with this solution is that oxalate inhibits prothrombin activation when present in excess.

5. Another method of fibrinogen removal was attempted by Gratia(1921) who used Staphylococci. The bacteria were found to act directly on the fibrinogen, the actual mechanism being unknown.

The definite source of prothrombin in the normal course of coagulation is still uncertain. At first it was thought that prothrombin could be obtained from many sources other than the blood, such as the lymph glands or corneal tissue. Today, however, it is believed that prothrombin only occurs in the blood or lymph, and blood platelets are considered to be the probable source. In addition, some fairly good evidence points to megacaryocytes as the source of much of this substance in the bone marrow. The prothrombin does not come from any other elements in the marrow.

Brinkhous(1940) has summed up much evidence which links the formation of prothrombin to vitamin K. The vitamin, itself, does not possess prothrombic activity, but apparantly it activates prothrombin or causes its formation.

Mills(1927) has suggested that the long latent period

preceding normal clotting is due to changes preparing for prothrombin liberation in the plasma.

The nature of the reaction that takes place when prothrombin is converted to thrombin has not yet been adequately solved. There are only theories to work on and compare. One hypothesis holds that the prothrombin molecule consists of two thrombin groups, and activation consists of precipitating one of these groups as a modified thrombin, water insoluble and fibrinolytic, while the other remains in solution as normal thrombin.

Prothrombin is usually considered to be present in the plasma of circulating blood, and to possess the possibility of ready conversion to thrombin upon the action of calcium and tissue extract. However, the importance of these two substances is actually in great dispute, and a more detailed investigation of the conversion process will be presented under individual discussions of the two factors involved. This refers to the thrombin theories of coagulation, but it must be remembered that there are also non-thrombin theories which neglect prothrombin.

For example, Fischer(1934) regards prothrombin as a term for proteins of globulin nature in blood and tissues generally, which combine with a lipoid element in tissue extract. This combination, by changing the iso-electric point of the globulin proteins, allows for fibrinogen flocculation, as we have seen.

C. Calcium

The ability to prevent clotting by means of oxalates, citrates, and other agents which decrease the calcium ion concentration, and the restoration of clotting upon subsequent addition of calcium, shows the importance of this element in the process of blood coagulation.

Prothrombin will cause immediate clotting upon addition to a fibrinogen solution or an oxalated plasma, when prepared from a calcium free plasma. Spontaneous clotting may occur in time, however, and the addition of calcium always causes immediate clotting.

Thus, whatever change is undergone by prothrombin in its conversion to thrombin, it appears that this change may take place without calcium, although the latter very greatly accelerates the reaction. Calcium plays the role of a catalyser in accelerating a reaction that can take place spontaneously in its absence.

This point of view is challenged by some workers. Howell (1916-1917), for example, accepts calcium as an essential agent in prothrombin conversion. Ferguson(1938) has shown that calcium is the most important factor in the formation of thrombin from prothrombin. Cephalin(tissue factor) seems to be of a secondary importance, improving thrombin formation in increased amounts.

The majority of evidence, especially that presented by Hammarsten(1883), seems to prove that calcium is not necessary

for the clotting of fibrinogen by thrombin. The tissue factor which acts on fibrinogen directly(Mills and Guest, 1921), demands the presence of calcium for its functioning. This belief is favored by the majority of experimenters. Collingwood and MacMahon(1912) suggest that calcium is necessary in the conversion of prothrombokinase to thrombokinase.

It has been shown that calcium exists in combination with some of the serum colloids, and also in ionized form. Some workers hold that the combined form is transformed to the ionized state during coagulation, and that the combined form is essential to clotting, being necessary for commencement of the process. Others, such as Ferguson(1936), believe that ionized calcium is essential for coagulation, forming thrombin from its inactive precursors.

Mellanby(1908) has shown that calcium possesses quite a specific coagulative property. He further has stated that temperature increases speed up fibrinogen conversion to fibrin, but this is not necessarily because calcium works by ionic properties. It is probably due to a faster breakdown of fibrinogen solution stability.

Scott and Chamberlain(1934) verified previous work that it takes about three times the amount of oxalate to prevent the coagulation of blood than would be expected from the total amount of calcium in the blood. Also, upon recalcification, fibrin forms even where there is a great excess of oxalate

present. This problem alone makes it clear that the relationships of calcium are by no means simple.

Stuber and Lang(1930) attempted to explain this phenomenon by concluding that the oxalate forms some sort of a salt complex with fibrinogen, and that the resulting high degree of ionization and dispersion of fibrinogen makes its precipitation impossible.

D. Tissue Factor

It has been known for a long time that a substance may be extracted from the tissues or platelets which greatly facilitates clotting. Mills(1921) showed that lung extract, for example, would cause death if introduced intravenously. He assumed that the death was due to intravascular clotting. He further showed that different tissue extracts have varying strengths, with the lung being strongest, then kidney, heart, brain, spleen, thymus, testis, and skin. The strong coagulative activity of lung and kidney tissue might be a suggestion of a protective mechanism against hemorrhage.

Actually, if tissue extract is injected into the blood stream in large enough amounts, clotting results. In smaller amounts the blood in rendered non-coagulable, partially or completely, due to removal of fibrinogen from the plasma, and final excretion via the urine.

Loeb(1907) demonstrated the presence of a tissue factor, and showed that it acts directly on fibrinogen, is not heat stable, varies the speed and quantity of coagulation according to the amount present, and is specific in regard to its action on plasma elements. He further considered the tissue factor to be a protein, and when other workers found that lipoid substances were contained, he modified his ideas and assumed a combination of protein and lipoids.

The extract may be obtained from fresh tissue by means of water or saline solutions, and from dried tissue by means of

alcohol. The former extracts are generally more potent, and are thermolabile, while the latter are not affected by boiling. The active constituent is generally agreed to be the same in both forms of extract and has been given various names such as thrombokinase, thromboplastin or thromboplastic substance, zymoplastic substance, cytozyme, cephalin protein compound or cytozymphosphatid.

Collingwood and MacMahon(1912) have stated that prothrombokinase is present in blood platelets, and is the precursor of thrombokinase. They further claim that platelet changes in shed blood allow the prothrombokinase to be acted upon by calcium to form thrombokinase. The insufficiency of evidence on this subject has left the issue undecided.

An argument arises as to whether the tissue factor is identical with the blood coagulins. Much evidence supports the view that they are different, especially since it is agreed that blood coagulins do not show those in the tissues.

Early workers assumed that the tissue factor was of lecithin nature, since it could be extracted from tissues by alcohol or ether, and besides being thermostabile in this form, it also contains phosphorus.

Howell(1912) showed that lecithin from egg yolk has no thromboplastic action. However, when he tested yolk itself, which contains small amounts of a phosphatid corresponding to cephalin, thromboplastic properties were noted. He also observed that ethereal solutions of dried brain or thymus also contain a

phosphatid, like cephalin, which has thromboplastic power. The liver and heart are also containers for this substance. Moreover, this phosphatid exerts its thromboplastic effect upon blood plasmas by neutralizing the action of the contained antithrombin.

Gratia and Levene(1922), and Wadsworth, Maltaner and Maltaner(1930) likewise found that pure lecithin has no coagulative properties. The latter, however, observed that a fraction might be obtained from pure lecithin which will cause coagulation. This fraction is very closely allied to the active lipoid substance obtained from tissue lipoids, and is believed to possibly be a small residue of cephalin. This tissue factor, therefore has come to be quite closely associated with cephalin.

Cephalin preparations lose their potency upon standing in the air, just as do purified preparations when they are reduced by the action of nascent hydrogen. It was then assumed, as a result, that cephalin's action on coagulation is due to unsaturated fatty acids in its molecules.

McLean(1917) was very enthusiastic in this belief. He said that cephalin is thromboplastic in relation to its degree of unsaturation. The more saturated it is, the less active it will be, and vice versa. Thus, cephalin is very potent when first isolated from tissues in coagulation, but soon, when saturated beyond a certain degree by oxidation or reduction, it loses this power. He further stated that cephalin, saturated,

or partly so, might retard coagulation by an acid reaction.

Evidence in support of this idea was found when cephalin was found to lose none of its potency in a vacuum. On the other hand, lecithin, even in forms which contain unsaturated fatty acid groups, has no thromboplastic property. Reduced cephalin is difficult to obtain, although there is good evidence to prove that it does exist. It is called hydrocephalin in this form.

Based on assumptions for the most part, rather than chemical determinations, is the idea that cephalin exists in some combination in the tissues, possibly as a compound with protein. This is more potent than cephalin, itself, and is thermolabile. Furthermore, it is assumed that such a complex also exists in blood platelets, and accounts for their effect on coagulation when they disintegrate. Another view on cephalin activity holds that ordinary preparations of cephalin possess thromboplastic properties due to the existance of some unknown impurity, or to a complex of cephalin and cerebroside. Still another report establishes the presence of a thromboplastic substance in the lungs which is not cephalin, although it is a lipoid related to the fatty acids. It is called anti-heparin, is thermostabile, and is of unknown chemical nature.

There are differing opinions in regard to the relationship of the tissue factor to prothrombin, and some people do not even accept the idea that it acts on prothrombin at all. Others assume that there are a few different tissue factors, which act

in different ways. Some of the divergent views in regard to the action of the tissue factor will now be summarized:

1. The tissue factor functions as a kinase or co-enzyme, which converts prothrombin to thrombin(Morawitz, 1904).

2. The tissue factor is a lecithin compound called cytozyme, which unites directly with the prothrombin(serozyme) of the blood, in the presence of calcium, to form thrombin(Bordet, 1921).

3. Cytozyme, and enzyme supplied by tissues or leucocytes, activates prothrombin(Fuld and Spiro, 1904).

4. The tissue factor forms fibrin by direct action on blood(Wooldridge, 1893). This author does not accept thrombin in his theory, and believes that the tissue factor clots blood in a different manner from thrombin. This is shown by clotting in vitro and in vivo, whereas thrombin only clots in vitro. The importance of the tissue factor in normal clotting, when blood escapes from vessels into the tissues, is thus shown.

5. The tissue factor is a protein cephalin compound, which forms fibrin upon chemical union with both calcium and fibrinogen of the blood(Mills, 1921).

6. The tissue factor(fibrinogen), after it has been used to coagulate blood, can be only partly extracted from the fibrin. It normally does not act by removing antithrombin, as Howell claims. On the other hand, the tissue extract will neutralize antithrombin if it is present. This strengthens the theory of Mills(Mills and Guest, 1921).

7. The purified tissue extract will not cause clotting if mixed directly with calcium and purified blood fibrinogen. The opposite results are due to contaminating prothrombin. This is contrary to the preceding idea(Smith, Warner and Brinkhous, 1934).

8. The tissue factor is a cephalin compound, which unites with the heparin or anti-prothrombin of the prothrombin-anti-prothrombin complex. Freed from this complex, the prothrombin may become activated to thrombin by the calcium in the blood (Howell, 1912).

9. The tissue factor is a protein phospholipid compound, which causes dissociation of the colloid complex of prothrombin, fibrinogen, blood globulin, and albumin, present in the circulating blood. The free prothrombin can then be activated to thrombin(Pickering, 1925).

10. The tissue factor contains two constituents which function differently. One is a cephalin compound which causes the prothrombin to be released from the prothrombin-anti-prothrombin complex, either by combining with it or neutralizing it. This same constituent then activates the free prothrombin to thrombin with the assistance of calcium. The other constituent is similar to blood prothrombin, and when activated it exerts the same effect upon the blood fibrinogen(Fuchs, 1930).

11. The tissue factor is a lipoid complex of alcohol soluble cephalin and cerebroside, which combines with prothrombin and calcium to form thrombin. The thrombin is then defined as

a calcium containing lipo-protein(Fischer, 1935).

12. An inactive enzyme in the plasma is activated by blood shedding or colloidal disturbance to become a "Thrombo-plastic" agent. This then converts prothrombin to thrombin in the presence of ionized calcium and cephalin(Ferguson, 1943).

E. Blood Platelets

Hayem(1877, 1822) and Bizzozero(1882, 1883) are given credit for discovering the existence and reactions of the blood platelets. Hayem demonstrated their existence in the blood, and described the rapidity of agglutination and disintegration that took place upon removing them from their natural enviroment. Bizzozero proved that, contrary to earlier opinions, thrombin in shed blood did not come from the disintegration of leucocytes, and that leucocytes actually undergo little or no change during the clotting period. Data obtained by both these men thus showed conclusively that platelet reactions from a preliminary step in the clotting of mammalian blood, and are the cause of large granular masses observed in newly shed blood. They further demonstrated that platelet agglutination is the initial phenomenon in thrombus formation.

On the basis of the work of these men it was generally agreed that platelet disintegration releases something into the plasma which precipitates the act of clotting, and that normally some anti-coagulant substances found in the blood preserves the platelets from destruction.

There is some question, however, as to whether platelet disintegration via foreign surface contact initiates clotting, or whether some unknown change in the plasma causes platelet disintegration. Whichever is the true case, there can be no doubt that platelets furnish some material upon disintegration that accelerates clotting and probably is essential in activat-

ing prothrombin to thrombin.

Doubt as to the primary reaction is justified when one realizes that in blood made incoagulable by manganese salt, the platelets disintegrate, but very slowly. On the other hand, certain organic peroxides preserve the platelets, but do not prevent coagulation. Some observers believe that surface phenomena aid considerably in platelet breakdown.

The exact chemical nature of the material liberated by the injured platelets is not known, although it has been shown that they contain lipoid material in addition to their protein constituents. This lipoid material, according to some evidence, is in part at least a phospholipid, which some workers assume to be lecithin, and others cephalin. Some other doubtful investigations have shown that a nucleoalbumin of importance to clotting may also be obtained from them.

Bayne-Jones(1912) has shown rather conclusively that disintegration of platelets aids blood clotting in two ways. Firstly, it sets free prothrombin, which is then activated to thrombin in the presence of calcium. This agrees with the views of Morawitz. Secondly, it liberates a thromboplastic substance which neutralizes the antithrombin normally present in the blood. He also refuted the idea that platelets contain all the essentials for clotting, an idea which would have challenged the views on fibrinogen. He found no clotting in extracts and emulsions of washed platelets in either water or saline solutions. Plasma was proved to be absolutely essential.

More recently, evidence has suggested that platelets are probably not the limiting factor in coagulation. It has been shown that platelet-free plasma has coagulative ability, and even Howell(1939), who firmly believed in the platelet disintegration hypothesis, now believes in the existence of a plasma thromboplastin. A final important function of the platelets is that they are responsible for clot retraction.

F. Anti-coagulants

There are three general types of anti-coagulants: fibrinolytic substances within various organs, substances foreign to the body, and substances in the circulating blood. For this thesis on coagulation, only the substances in the blood, which include heparin, antithrombin, and antithrombokinase, will be dealt with.

It has been proved that blood serum may delay blood clotting, and this plus other experimentation has led to the important concept that heparin is present in the blood. Heparin is widely distributed throughout the body, although its greatest concentrations are found in the liver, lungs, and muscles. According to Charles and Scott(1933), and some other modern evidence, mast cells are the possible source of this substance. According to Quick(1936-37), especially since much higher concentrations have been found in the tissues than in the blood, there is no good reason to assume that heparin is a constituent of the blood.

The heparin unit is the amount of heparin required to prevent the coagulation of 1 c.c. of cat's blood for 24 hours, and its concentration is usually determined in this unit. Its dissociation constant is 2.0×10^{-4}; no methods have been found yet, which are sensitive enough to determine its absolute concentrations in blood or tissues.

As in the case with most materials involved in blood coagulation, heparin's chemical nature is not well agreed upon by

all workers.

Howell(1923) reported that it was an amorphous water soluble substance, without nitrogen, but with phosphorus, and a carbohydrate group. Later(1928) he considered it as either a paired glycuronate or a condensation product of glycuronic acid.

Today, due to the work of Charles and Scott(1933), phosphorus and nitrogen are believed to be present, and Jorpes(1935) added the idea of the presence of calcium and sulphuric acid residue. Jorpes went even further, and advanced the theory that heparin is a chondroitin polysulphuric acid. This is the basis of all recent views on the chemical structure of heparin. He also stated that hexuronic acid and hexoseamine are present in the proportion of one to one.

Schmitz and Fischer(1933) believed that they isolated heparin in the form of a white, water soluble, nitrogen free powder. The formula, $C_{18}H_{32}O_{17}$ $6H_2O$, was even assigned to this compound.

Uronic acid is present, and many workers believe, as a result, that heparin is a carbohydrate compound which contains one free carboxyl group, and which therefore acts as a monobasic acid. Charles and Scott have mentioned a free carboxyl and a free NH_2 group, which cause it to react like an amphoteric substance. They refuted the uronic grouping, but confirmed the carbohydrate portion. They also said that it did not give a test

for protein or glycogen, was destroyed by pepsin, trypsin or distase, was readily soluble in acid and alkali, and did not distil at 175 degrees C., at a pressure of .001mm. of mercury.

Howell(1923) and Fischer(1935) conclude that the uronic acid residues are responsible for heparin activity. Jorpes believes that it is the sulphate groups. A safer view point is that the anticoagulant activity depends upon all the groups in the molecule, and their appropriate arrangement. Further study is required to determine the exact chemical nature of heparin, and the molecular grouping which controls its anticoagulant effect.

Heparin has been found by several workers to occur normally in the plasma, but its presence in serum has never been observed. Howell(1924-25) gives as a reason the fact that heparin is in combination with prothrombin, and by inactivating it, maintains the fluidity of the blood. He reports that the addition of heparin to a mixture of prothrombin, calcium, and tissue factor prevents thrombin formation. Opposite results have also been reported, and the explanation of the conflict probably lies in the differences in reagents used.

Howell further maintains rather firmly that heparin will not prevent the reaction between pure preparations of thrombin and fibrinogen, and is thus not an antithrombin, but an antiprothrombin. His argument against views that heparin has an antithrombin action is the questionable purity of the reagents involved.

There is indecision, therefore, in regard to whether heparin is antithrombic or antiprothrombic. It is usually considered to be both, with some unknown substance in blood necessary for its respective actions.

Quick(1936) makes use of this idea in suggesting a heparin action. He says that heparin is neither an antithrombin nor an antiprothrombin, but is an antithrombogen. This is an agent which reacts with a constituent in the plasma to form a true antithrombin. He bases this idea on the fact that heparin will not prevent pure solutions of prothrombin or fibrinogen from clotting.

This reaction fails if the plasma is heated to 70 degrees C. The explanation given is that there is some sort of a reaction between heparin and the salts in the blood. Removal of the salts by dialysis halts the reaction, but no account is thereby given for failure of the reaction upon heating. The substance with which the heparin reacts has been termed pro-antithrombin, and will be further described under a discussion of antithrombin.

A third idea of heparin activity is that it combines with, and neutralizes the tissue factor, thus exerting its anticoagulant effect. This theory is based on the fact that trypsin will destroy the anticoagulant action of hirudin, but not that of heparin. This indicates that heparin, unlike hirudin, is not a proteolytic substrate, and therefore cannot react with thrombin, which is regarded as a proteolytic enzyme of the nature oftrypsin.

Mellanby(1934) has proved that heparin is more abudant in tissues than in the blood. He therefore believes that heparin maintains blood fluidity by continually passing into the blood stream to neutralize the kinase from tissue breakdown. Best, Cowan and MacLean(1938) have shown that heparin inhibits cellular agglutination, and also functions as an anti-plasma coagulant.

At the present, there are believed to be various species of heparin with different heparins existing in each.

Antithrombin is a substance of unknown chemical nature, which normally occurs in small concentrations in blood plasma, serum, and lymph, and either combines with or neutralizes active thrombin. No conclusive evidence has yet been obtained to determine whether or not it affects prothrombin in a similar manner. After the initial studies of Morawitz, it has been investigated by numerous workers.

Some literature points to antithrombin formation as arising from the liver. Other reports claim that antithrombin comes from the action of heparin on a substance in the blood called pro-antithrombin. Howell is a firm believer in the latter. He discovered that the addition of heparin to the blood resulted in an increased antithrombin concentration, and he attributed it to increased pro-antithrombin conversion.

Quick(1938) has a different idea as to how antithrombin action increases upon heparin addition. He believes that antithrombin is an albumin, and fibrinogen has a greater attraction

for thrombin than albumin has. Thus, no inactivation of thrombin results until all the fibrinogen is converted to fibrin. The addition of heparin, however, increases the albumin affinity for thrombin, so that thrombin combines with the heparin-albumin complex before it reacts with fibrinogen.

Pro-antithrombin, as we have seen, is destroyed by heating to 70 degrees C. It may be precipitated from plasma by acetic acid or by ammonium sulphate added to one half saturation. It is supposedly present as a normal constituent of the blood, to aid in maintaining its fluidity. That antithrombin is normally present in plasma, serum, and lymph, has been shown by several workers.

Mellanby(1908), for example, slowly injected large amounts of thrombin into a cat without inducing intravascular coagulation, and the result was interpreted in terms of anti-thrombin. Also, he showed that serum contains practically no thrombin after standing for one hour following coagulation.

Collingwood and MacMahon(1912) added additional evidence when they mixed oxalated blood and thrombin, and tested the activity at various intervals. They found that rapid destruction of thrombin takes place in the blood, and the irreversibility they find in this reaction aids them in assuming that a true antibody is present, and not more adsorption of thrombin by the proteins present.

Schmidt(1893, 1895), on the other hand, recognized this substance as being present in serum, and managed to get active

thrombin from the result of the thrombin-antithrombin reaction.

The inactivated thrombin is called metathrombin, and as suggested by Schmidt it is generally believed to be a compound of thrombin and antithrombin, with the reaction being a reversible one. Alkali activation may liberate thrombin by destroying antithrombin, and the metathrombin fromation is probably protective in removing any free thrombin which arises in normal circulation.

Rich(1917) has confirmed the idea that metathrombin results from a combination of thrombin and antithrombin, and has attempted to prove it by presenting the following facts:

1. Metathrombin cannot be produced in solutions lacking either thrombin or antithrombin.
2. Metathrombin is readily formed if these two factors are present.
3. Thrombin and antithrombin gradually decrease in amountsin solutions where both are present.
4. No other known coagulative factors will cause metathrombin formation.

Collingwood and MacMahon(1912) have shown the probability of an anti-thrombokinase as being present in the circulating blood. Testing for the possible disappearance of thrombokinase from blood serum, they discovered that it disappeared rapidly at first, and then after a period of quiescence there came a slow gradual loss.

The explanation given for the rapid disappearance was

that a true anti-thrombokinase was functioning. This fact was supported by failure at reactivation attempts. The slow disappearance of thrombokinase was ascribed to protein adsorption. In this case the thrombokinase could be reactivated.

G. Proteolytic enzymes

The existence of enzymes in blood plasma has been suspected for a long time, but their role in coagulation is obscure. It has never been integrated with all the known facts on coagulation. Many workers had shown that normal blood serum could digest gelatin and casein, and could also split proteins, if treated with chloroform. This suggested the presence of an enzyme in the plasma, but arguments arose that cellular elements had not been excluded, and were the source of the enzyme. These arguments were stopped by Hedin(1903), however, who obtained similar results with cell-free ox plasma. Thus, the existance of a plasma enzyme was more readily accepted, although its relationship to coagulation was not, and still is not understood. Hedin further stated that the enzyme inactivity in plasma was due to antibodies, and that the easiest way to obtain it was by precipitation with a nuclein substance like casein.

Tagnon, Davidson and Taylor(1942) converted globulin substances and plasma euglobulin, which have no proteolytic activity, into globulin fractions with marked proteolytic activity, by the use of chloroform. This and other evidence reveals that an enzyme will not work until activated somehow, either physically or chemically.

Either crude or crystalline trypsin will cause blood to coagulate (Eagle and Harris, 1936-37). It does not clot fibrin-

ogen directly, but forms thrombin when it reacts with prothrombin. It thus has the same effect as calcium plus tissue extracts, and suggests that the latter system might contain a proteolytic enzyme with a specific affinity for prothrombin. This also tends to show that calcium is not an intrinsic part of thrombin.

Actually, in the action of trypsin there is no sufficient evidence as to whether the prothrombin is hydrolyzed, or whether the enzyme combines with the prothrombin to form a modified enzyme, thrombin.

Papain is another enzyme which will also initiate clotting, but in this case it is of a different mechanism. The papain is like a true thrombin in that it acts directly on fibrinogen to form an insoluble substance, which is almost exactly like fibrin. The resemblance of both clots is fairly strong evidence that thrombin is a proteolytic enzyme with specific action on fibrinogen.

Tagnon(1941-42) has attempted to show that a proteolytic enzyme exists in plasma, which is active in blood coagulation, and changes prothrombin to thrombin without calcium, thromboplastin or platelets, in the manner of trypsin. Prothrombin, he further adds, counteracts the proteolytic action of the enzyme on fibrinogen, and is assisted by thromboplastin, which by itself does not have this power. Prothrombin directs the enzyme's action to a clotting effect. Since the enzyme comes from the plasma and forms thrombin

without the intervention of calcium or thromboplastin, it appears quite possible that the enzyme plays a primary role in the mechanism of normal blood coagulation.

Before the enzyme role in coagulation can be accepted, however, several questions must be answered. There must be an explanation for the activating action of chloroform and other agents, and the condition of the enzyme in the blood. The method of control for clotting or lysing must be understood, and above all, the knowledge of enzymes must be integrated with the known facts on coagulation.

H. Non-Thrombin Theories of Plasma Coagulation.

Several theories on blood coagulation have omitted the use of thrombin, although excellent proof has been given that its addition to a fibrinogen solution will result in fibrin formation, and deposition of a clot. Workers along these lines argue that this reaction is not found in normal blood clotting, or at least is not the essential change.

According to Freissly(1943) the classic theory of blood coagulation, in which prothrombin is activated upon platelet breakdown, must be seriously doubted. He has obtained a prothrombin activator from the plasma, which differs from that in platelets. Therefore, he believes that although platelet-disintegration products may accelerate coagulation, other factors are concerned in the initiation of the process.

Another view holds that plasma coagulation is a physicochemical reaction dependent on a change in the surface forces in the colloid system. This explanation is even less generally accepted than the non-thrombin view. Most workers, today, accept variations of the thrombin theory as most valid in describing plasma coagulation, nd their views will be presented under a discussion of the thrombin theories.

Wooldridge(1893) held to the non-thrombin view of clotting. He said that clotting was the result of a reaction between two substances(A and B), each consisting of a protein combined with lecithin or a lecithin-like body. A-fibrinogen is found in blood

and tissues, and B-fibrinogen is found only in blood. The exact nature of the reaction was not specified, except to claim the importance of lecithin in producing a change. Mills and Guest(1921) agree with Wooldridge's idea of two fibrinogens reacting, and consider this to be the primary reaction in normal clotting, although they recognize the existance of thrombin.

The general weakness of these theories is illustrated by failure of tissue extract to successfully clot a solution of fibrinogen and calcium. Non-thrombin believers defend themselves against this fact by assuming a change in the fibrinogen upon removal from plasma.

Stuber and Lang(1930) refute the importance of calcium as well as that of thrombin. Their idea is that blood glycolysis is the ultimate cause of clotting. Lactic acid increases the hydrogen ion concentration in the blood, and this precipitates the fibrinogen. This idea is weak in that such fibrinogen precipitation is reversible, unlike the normal clotting reaction. Also, the characteristic gel structure is absent.

Nolf(1906) states that three colloidal substances unite to form fibrin. Two are protein bodies, fibrinogen and thrombogen, while the third, thrombozyme, is a proteolytic enzyme. Thrombin, a combination of these three substances, is therefore actually a soluble form of fibrin. This is not a thrombin theory, since the central theme is proteolysis.

I. Classical Theories of Plasma Coagulation

The classical, or thrombin theories of coagulation are based upon four fundamental facts.

1. The development of thrombin in shed blood, which is present as prothrombin in the circulating blood, is the cause of clotting.
2. Thrombin plus fibrinogen react to form fibrin
3. Normal clotting depends upon the presence of calcium.
4. Thrombin formation depends upon a substance released from tissue cells.

1. Morawitz's Theory(1904)

Morawitz was the first man to combine these facts successfully. His theory is as follows:

Prothrombin(Thrombogen) x Calcium x Thrombokinase = Thrombin

Thrombin x Fibrinogen = Fibrin

The tissue factor is an organic kinase arising from platelet disintegration, and also from tissues, especially if they are wounded. Prothrombin, calcium and fibrinogen are present in the circulating blood, with prothrombin inactive in the absence of the kinase.

2. Bordet's Theory(1912)

Proserozyme x foreign contact x calcium = serozyme

Serozyme(prothrombin) x Cytozyme x Calcium = Thrombin

Thrombin x Fibrinogen = Fibrin

The platelets furnish cytozyme, a lecithin tissue factor-not a kinase. Serozyme is in the inactive form of proserozyme in circulating blood, probably due to an inhibiting substance, which some physico-chemical reaction releases. Serozyme is thermolabile, unlike cytozyme which may be heated above 100 degrees without losing its properties.

3. Howell's Theory(1918)

Prothrombin-antiprothrombin x Tissue Factor = free prothrombin

Prothrombin x Calcium = Thrombin

Thrombin x Fibrinogen = Fibrin

Howell was the first author to assume the presence of an inhibiting substance(heparin) in the blood, which prevents coagulation. Heparin plus prothrombin gives a prothrombin-antiprothrombin complex. The tissue factor, from platelets or injured tissue, combines with the antiprothrombin(heparin) to release free prothrombin. The tissue factor is a cephalin compound. Additional prothrombin may come from platelet disintegration.

4. Pickering's Theory(1925)

$$\text{Prothrombin-Fibrinogen-Blood Proteins} \xrightarrow[\text{(tissue factor)}]{\text{(foreign surface)}} \text{free Prothrombin}$$

Prothrombin x Calcium (x Tissue Factor) = Thrombin

Thrombin x Fibrinogen
or = Fibrin
Tissue Factor x Fibrinogen

There is a physico-chemical combination of prothrombin, fibrinogen, and the blood proteins(serum globulin, serum albumin). Prothrombin is thus kept inactive. Free prothrombin is the result of the complex breaking down, either by foreign contact, or by action of tissue extracts.

If the complex is broken down by physical contact, calcium converts prothrombin to thrombin. If the tissue factor does this, it goes on to aid prothrombin conversion. In any case, the thrombin reacts with fibrinogen to form fibrin, once it has itself been formed. Sometimes, there may even be a direct reaction between the tissue factor and fibrinogen to form fibrin.

5. Fischer's Theory(1935)

Prothrombin x Calcium x Thrombokinase = Thrombin

Thrombin x Fibrinogen = Fibrin

Prothrombin is a globulin occuring in both tissues and blood plasma. It is changed to thrombin in both places. The tissue factor, thrombokinase, is a lipoid complex of an alcohol soluble form of cephalin and a cerebroside. Thrombin is a calcium containing lipoprotein.

Thrombokinase affects prothrombin in two ways. Firstly, it modifies prothrombin to become isoelectric near its neutral

point; non-specifically. Secondly, by some specific effect upon the modified prothrombin(thrombin) the kinase makes possible the coupling of the thrombin with fibrinogen.

Heparin is accounted for by assuming that it unites with prothrombin. This changes the isoelectric point of the prothrombin towards the acid side, and the specific kinase effect is stopped.

6. Schmidt's Theory(1893)

Prothrombin x Zymoplastic substance = Thrombin

Thrombin x "Fibrinoplastic" substance x Fibrinogen = Fibrin

The Zymoplastic substance, or tissue factor is a lecithin compound which comes from the platelets. The fibrinoplastic substance aids the thrombin action on fibrin; and is present in the platelets also. It counteracts the effect of an inhibiting substance, cytoglobin, which is an antithrombin.

7. Ferguson's Theory(1943)

Inactive Tryptase = Active Tryptase

Tryptase x Calcium x Prothrombin x Cephalin = Thrombin

Thrombin x Fibrinogen = Fibrin

Tryptase in the blood is activated by blood shedding or colloidal disturbance. Cephalin is the tissue factor. Thrombin functions as another enzyme in forming fibrin from fibrinogen.

8. Hekma's Theory(1931)

Fibrinogen, Prothrombin, Globulin & Albumin x Calcium x Kinase = Free Globulin, Albumin, Fibrinogen and Thrombin

Thrombin x Fibrinogen = Fibrinogen Filaments

Proagglutinin x Calcium = Agglutinin

Agglutinin x Fibrinogen Filaments = Fibrin

Kinase comes from the leucocytes and platelets. Kinase releases free fibrinogen from the complex. The pro-agglutinin comes from the red cells. Calcium functions to form agglutinin from pro-agglutinin.

IV Thrombus Etiology

The ultimate cause of intravascular clotting still remains a mystery, but it is generally agreed that multiple factors account for the complex origin.

There are, in general, three main etiological factors which are known to have some bearing upon this phenomenon, and they are considered to be quite important. These factors are influenced by conditions which have long been a source of speculation.

A. Alteration in the condition of the vessel wall

B. Alteration in the blood composition

C. Alteration in the rate of circulation

In the past, chemical and physical hypotheses have alternated at different times. Today, biochemical and physico-chemical viewpoints are receiving the attention of the majority of workers. In the following pages more detail will be given of the three items listed above, along with other contributory or predisposing factors which operate through these main channels.

Actually, as suggested above, and as Knisely and workers (1943) suggest, "There has, however, been no rigorous, systematic search for all the biological, physical and chemical etiologic agents capable of initiating intravascular agglutination of the blood."

A. Alteration in the condition of the vessel wall

Arguments have been set forth continually in regard to the importance of this effect upon thrombosis. It is agreed by many workers that severe injury to the intima will result in thrombosis, while the other two factors cannot function alone in this respect(Frykholm, 1940). Injury to vessel walls may come from factors within or without the vessel. There are differing views as to how injured vessels bring about thrombosis. The most simple idea is that the injured spot serves as a "foothold" for elements.

Injury to the endothelium is associated with the formation of white cell thrombi, and Frykholm(1940) said that when the vessel wall is injured, cells become roughened, or fall off, and thrombocytes or leucocytes may adhere to them. Leucocytes travel along vessel walls, as do the thrombocytes.

On the other hand, a thrombus which has the appearance of an extravascular clot is formed in those cases of intravascular clotting in which the endothelium is more or less intact. In such cases thrombosis occurs due to stasis in the vessel, together with other factors which favor coagulation, and red thrombi result.

Clark and Clark(1935) have experimentally shown that changes take place in endothelia, and that the resulting stickiness causes leukocytes to attach. This endothelial change occurs normally to some degree, and especially in injury.

Altschule(1942) and others have speculated that the plate-

let thrombi are secondary to damaged vascular endothelia. On the other hand, Bernheim(1943) and others believe that the endothelial damage is secondary to the presence of platelet thrombi.

Gortner and Briggs(1928) do not believe that a "wettable" surface causes coagulation by the clumping of elements. They rather believe that there is an establishment of suitable electrical forces between the blood and the contact surface, and this accounts for foreign surface activity. They proved by electrical measurements that a glass surface tends to adsorb positively charged colloids much more favorably than a paraffin covered surface. They then came to the conclusion that the initial step in blood-clotting involves a surface concentration of a positively charged constituent, brought about by the selective adsorption.

Another another approach to thrombus etiology has been proposed by Ritter(1926). After the layers of vessel walls are irritated, this author believes that they disintegrate, changing the hydrogen ion concentration. The resulting colloid chemical change in the layer between blood and tissue is considered essential to intravascular clotting. The physico-chemical equilibrium associated with changes of blood viscosity is altered, the vessel wall adsorbing the cellular elements, or precipitating fibrin.

Taylor(1939), and Lozner, Taylor and MacDonald(1942) have given evidence which would tend to claim that foreign surface contacts do not affect cellular elements, but probably cause a

physico-chemical change in the cell-free plasma.

Taylor has shown that paraffin surfaces do not delay blood clotting by preventing destruction of platelets, since in platelet-free preparations the results obtained show foreign surface effects to be just a great. He thus referred to the importance of what he terms the globulin substance.

Lozner, Taylor and MacDonald came to the same conclusion when they observed that there was no lysis of platelets after plasma containing a great many of these elements was exposed to foreign surfaces for one hour. They suggest that the plasma euglobulin fraction is at least one of the factors which is modified. Howell called this substance "Plasma thromboplastin."

Thus, the intima injury may increase the globulin-fibrinogen fraction of the blood plasma, resulting in increased amounts of coagulation-accelerating substances, and an increased coagulation of the plasma, together with an increased possibility of agglutination.

A passive view on agglutination has been proposed by Klemensiewics(1919), after injury to the vessel wall. He has stated that in the mesenteric vessels of salamanders a fibrous, gelatinous membrane is laid down on the injured endothelium. This delicate mass of threads, filaments or bands attaches to the intima, and entangles the spindle cells(Amphibian platelets) as they pass by. Thus, according to this author, the spindle cells are not the progenitors of fibrin threads, and are passive performers

in thrombosis.

An outstanding flaw in this theory is its conflict with the established idea that agglutination is generally the primary step in thrombosis. Also, he has no explanation for the fact that heparinized blood can still form clots.

B. Alteration in the Blood Composition

Alteration in the composition of the blood, either chemical or physical, is definitely believed to take part in thrombosis, as suggested. There are several clinical conditions which may alter the coagulability of the plasma, besides tissue injury. It may be said , in general, that any process which tends to liberate coagulins may cause thrombus formation or add to an already agglutinated mass. Coagulins may come from disintegrated, agglutinated thrombocytes, red corpuscles, injured vessel walls or nearby tissue, or from previously formed fibrin. Products from microorganisms might also cause thrombosis, by some chemical action which is not yet understood.

We have already spoken of the increased globulin-fibrinogen fraction in the blood, which is associated with decreased blood albumin. This biochemical viewpoint is receiving a great deal of attention by modern workers. Frykholm(1940) has said that the alterations in blood consist of an increase in the globulin concentration of the blood plasma, in proportion to the albumin concentration. This causes the colloidal equilibrium of blood plasma to become less stable. Besides causing plasma coagulation which usually adds on to agglutinated cells, this change in the globulin-fibrinogen fraction may also result in agglutination.

The role of platelets in intravascular clotting is a very confusing one, and its importance has caused much argument. Some workers have attempted to show experimentally that platelet breakdown initiates clotting. Others believe that the plasma changes,

and causes platelet disintegration.

Mackay(1931) has refuted the idea that a superabundance of platelets necessarily results in thrombosis. Others have argued, however, that an excess of circulating platelets probably does contribute to rapid growth of a thrombus, especially where there is a laminated structure of platelets and clotted blood.

The latter view is more acceptable, since it is obvious that platelets possess a material which aids plasma coagulation. Johnson(1932) has supported the view that fibrin comes from platelet products, by studies on experimental white thrombi in which fibrin was observed at very early stages. Similar observations were made by Apitz(1939), who was agglutination of platelets, and then fibrin, when he used precipitating solutions of profibrin.

Thus, it seems very natural to assume that in intravascular clotting there is an agglutination of platelets, along with fibrin deposition caused by material from injured platelets.

C. Alteration in the Rate of Circulation

In regard to the older views on mechanical formation of thrombi, work was done on obstruction of water flow. The observed whirls, eddies and changes in rate of flow were also believed to occur in the blood stream from some obstruction, and to cause plasma coagulation or cellular agglutination.

Results from these experiments, however, do not fit in very well with the known facts on thrombus etiology, and their great significance has decreased, until today they are mostly important in the explanation of laminated thrombi formation.

Workers, by ligating off sections of blood vessels, found that no thrombi were formed in the isolated portions for quite some time, if the vessel wall was left uninjured(Frykholm, 1940).

Thrombi will eventually form where a stagnation of the blood occurs due to injury of the vessel wall by interference with nutrition. It was thus further assumed by Frykholm that blood stoppage is not sufficient cause for thrombosis. Passage of cellular elements along a certain part of a vessel is essential for thrombus formation. Since all thrombi have agglutinated elements as a core, a supply of these elements is essential.

Too rapid a flow is also of disadvantage for thrombus formation, since the first deposits of the thrombus will be washed away leaving no "foothold." The most favorable condition is that of a slower than normal current.

Summary and Conclusions

More fruitful advances in the study of intravascular clotting should be made with improved methods of microtechnique. At present, the conflicting, uncertain ideas are largely the product of inadequate methods of investigation.

The most striking feature in a study of this subject is the enormous accumulation of literature, most of which is filled with conflict. There are a multitude of experimental findings which seem to defy opposition, and yet there almost invariably arises some new material which presents a serious challenge to the original views. Correlation and selection of these "acceptable" facts is necessary before further light can be shed on the problem of thrombosis.

It appears that blood sludge will play quite an important part in future studies on thrombosis, especially in regard to the basic mechanism of cell agglutination.

Abstract

Intravascular clotting was originally considered to be the result of plasma coagulation. Later, however, with the discovery and study of blood platelets by Bizzozero, the importance of these factors in thrombosis was recognized. There was then introduced the concept that intravascular clotting is the twofold result of plasma coagulation and cell agglutination. These two processes are actually quite different, and they are taken up individually.

Phylogenetically, these processes developed together. Cell agglutination developed first, and is the only process present in more primitive organisms. Plasma coagulation was added in higher animals, and became more pronounced, although the agglutination process was retained. In these higher forms the agglutination is usually an initiating factor, and the fibrin deposition is then added, forming the major part of the clot. A pure fibrin clot, as in the case of a pure agglutination clot, is only rarely found among the more well developed species.

There are several theories as to cell agglutination, with the most acceptable one, by Loeb, assuming an amoeboid movement to be the initiating factor. Other theories are based on electrical surface changes, protein deposition on cell surfaces, and salt removal from the stromatic colloids of corpuscles.

The presence of agglutinated cells in plasma, following injury, has been investigated in the fairly recent studies on blood sludge. It is hoped that continued studies along these lines will aid in

unravelling the mystery of thrombosis, since there is such a definite relationship between the two subjects.

The three major types of thrombi are reviewed, namely agglutination thrombi, mixed thrombi and fibrin thrombi, the second type being by far most common. The agglutination thrombi may be further subdivided according to the preponderance or exclusiveness of the elements involved. There are thus platelet thrombi, white corpuscle-thrombi, red corpuscle-thrombi and mixed thrombi. Here again a pure thrombus is rare, and the mixed thrombi form the vast majority of such structures. The morphology of the thrombus is greatly influenced by the rate and type of blood flow.

Under the study of plasma coagulation there are but two facts which are quite generally accepted from among the accumulated mass of disputable material.

1. The formation of thrombin from its precursors; prothrombin, calcium and the tissue factor(thrombokinase).
2. The conversion of fibrinogen to fibrin under the influence of thrombin.

These facts form the basis for most modern acceptable theories on coagulation, the so-called thrombin theories, the most important of which are described, following an account of the agents associated with coagulation, and some non-thrombin theories.

The exact nature of the fibrinogen conversion to fibrin is still a matter of much dispute. Some evidence points to thrombin's action as that of an enzyme. Another theory holds that increased acidity affects fibrinogen, which is actually an alka-

lihydrosal of fibrin dispersed as amicrons. Electrical effects, fibrinogen complex dissociation, flocculation at fibrinogen's isoelectric point and chemical union are other suggested views as to the conversion process.

Good evidence has indicated a constituent of normal cell-free plasma, which may clot blood independent of tissue extract, prothrombin or fibrinogen, in the presence of calcium. The concept of this plasma euglobulin has not yet been reconciled with other known facts on coagulation.

Prothrombin is generally believed to be a protein, but further verification awaits the preparation of the substance in purer form. Megakaryocytes in bone marrow, along with platelets are considered to be the sources of prothrombin which is normally present in the circulating blood plasma. The mechanism for prothrombin conversion to thrombin is still very vague, although some ideas are mentioned.

There is much indecision as to whether calcium is necessary for prothrombin conversion to thrombin, and most evidence points to its not being essential in the clotting of fibrinogen by thrombin. Some workers believe that the ionized form of calcium is the active one.

The tissue factor is quite generally considered to be of a cephalin nature, and is present in platelets and tissues. There are often considered to be several types, all of which are different from the blood coagulins. Summaries are given of several theories on the action of this substance. One form of the factor

supposedly acts on prothrombin in its conversion, and the other acts directly on fibrinogen.

There is uncertainty as to whether platelet disintegration initiates clotting, or plasma changes cause platelet breakdown. Whichever is the true case, platelets undoubtedly furnish some material when they disintegrate, which aid blood clotting.

There are three anticoagulants known to be present in blood plasma. Heparin's function is disputed. It has been described as an anti-thrombin, antiprothrombin, and a combination of both. Another view holds that it exerts its effect by acting on a constituent of the blood plasma, while a final theory is that it neutralizes the tissue factor.

A true antithrombin is considered to be present in plasma, which increases in some manner upon the addition of heparin. An anti-thrombokinase is likewise believed to be present.

The enzyme presence and role in coagulation is one of the most uncertain points in regard to clotting. An enzyme appears to be present, but very few questions about it are answerable, and these are all dubious.

Surprisingly little is known of the ultimate cause of thrombosis. Its initiation appears to be very complex, although three main etiological factors have been mentioned, and a detailed account of their action is given. They are:

A. Alteration in the vessel wall condition

B. Alteration in the blood composition

C. Alteration in the rate of circulation.

Injured vessel walls usually result in white thrombi, while intact walls cause red thrombi formation. Injured walls and their results are given various interpretations. Stickiness, electrical changes, changes in the hydrogen ion concentrations and physico-chemical changes are used to explain their effect. A passive view in regard to resulting agglutination has also been given.

In regard to the change in blood composition, much attention is currently paid to the biochemical view which considers that there is an increase in the globulin-fibrinogen fraction of the blood. In fact, any process which tends to liberate coagulins may cause or add to thrombus formation. Coagulins may come from platelets, red corpuscles, injured vessel walls or nearby tissues, from previously formed fibrin and from products of microorganisms.

The optimum alteration in the circulation rate is that of a slower than normal current. Earlier workers spent much time on this mechanical viewpoint, but today it is receiving little attention.

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